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TESTING EXPERIMENTAL COMPOUNDS AGAINST AMERICAN MUCOCUTANEOUS AND CUTANEOUS LEISHMANIASIS

ANNUAL REPORT 2

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Leishmania donovani, L.m. mexicana, L. braziliensis panamensis, L.b.guyanensis

BALB/cByJ mice	Combination Chemotherapy	Topical Creams	WR 2975
BCG	difluoromethylornithine (DFMO)	Therapeutic Index	2116-66
Pentostam	Bleomycin	Pentostam Index	227-495
Ketoconazole	Hydrolyzed Ketoconazole	Aminoquinolines	219-423

20. ABSTRACT *(Continue on reverse side if necessary and identify by block number)*

Over a two year period under this contract, eight WRAIR compounds have been tested against two subspecies of L. m. mexicana, L. donovani, or L. braziliensis. In the visceral model, 6 of eight WRAIR exerimental aminoquinolines were as active as the standard antimonial Pentostam in decreasing spleen and liver parasite burdens, as shown by Pentostam Indices of 1.4 to 11.8. However, spleens from these mice were all culture positive so that no cures were achieved with them.

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Cutaneous and mucocutaneous infections were only slightly affected by experimental drug treatment. None of the Therapeutic Indices of these drugs was competitive with Pentostam (TI = 1.20 to 2.00 v/s 160). The LD50 of Pentostam was 600 mg/kg/day, whereas that for WRAIR experimental drugs was 10 to 125 mg/kg/day. Based upon these data, none of the WAIR aminoquinolines tested show promise for further developement.

Although ketoconazole and its acid hydrolysate were reported effective against Trypanosoma cruzi in vivo and in vitro, and against amastigotes and promastigotes Leishmania species in vitro, we find these two compounds inactive against L. donovani infections in BALB/c mice. Therefore, neither of these imidazoles shows promise for further development.

Three types of combination chemotherapy were tested against visceral, cutaneous, and/or mucocutaneous infections in BALB/c mice. These included systemic and topical application of Pentostam against L. braziliensis, Pentostam and bacille Calmette Guerin (BCG) against L. mexicana infections, and alpha D,L-difluoromethylornithine (DFMO) in combination with Bleomycin against L. donovani. Of the combinations tested, only DFMO (1% in drinking water) in combination with the antitumor drug Bleomycin (3 mg/kg/day) was competitive with Pentostam. Cures were achieved and parasite liver burdens were suppressed whether this combination was given before, at time of, or after infection.

This is the first time in 30 years a new drug which is non-toxic and specific for a parasite enzyme pathway has been identified against leshmaniasis. Its prophylactic effect and ease of delivery suggest that DFMO should be tested in combination with other known, active compounds eg. Pentostam and Pentamidine, and with new experimental ones eg. allopurinol riboside.

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SUMMARY

A. Experimental WRAIR Compounds: Aminoquinolines

Over a two year period under this contract, eight WRAIR compounds have been tested against two subspecies of L. m. mexicana, L. donovani, or L. braziliensis. In the visceral model, 6 of 8 WRAIR experimental aminoquinolines were as active as the standard antimonial Pentostam (Burroughs-Wellcome, Beckenham, England) in decreasing spleen and liver parasite burdens, as shown by Pentostam Indices (PI) 1.4 to 11.8. However, spleens from these mice were all culture positive.

Cutaneous and mucocutaneous infections were only slightly altered by experimental drug treatment. None of the Therapeutic Indices of these compounds was competitive with Pentostam (TI = 1.20 to 2.00 versus 160). The LD50 of Pentostam was 600 mg/kg/day (mkd), whereas that for WRAIR experimental drugs was 10 to 125 mkd. Based upon these data, none of WRAIR aminoquinoline compounds show promise for further development.

B. Experimental WRAIR Compounds: Imidazoles

Although ketoconazole and its acid hydrolysate were reported effective against Trypanosoma cruzi in vivo and in vitro, and against amastigotes and promastigotes of Leishmania species in vitro, we find these two compounds inactive against L. donovani infections in BALB/c mice. Therefore, neither of these imidazoles shows promise for further development. They will not be tested further.

C. Combination Chemotherapy

Three types of combination chemotherapy were tested against visceral, cutaneous, and/or mucocutaneous infections in BALB/c mice. These included systemic and topical application of Pentostam against L. braziliensis, Pentostam and bacille Calmette Guerin (BCG) against L. mexicana infections, and alpha D,L-difluoromethylornithine (DFMO) in combination with Bleomycin against L. donovani. Of the combinations tested, only DFMO (1% in drinking water) in combination with the antitumor drug Bleomycin (3 mkd) was competitive with Pentostam. Cures were achieved and parasite liver burdens were suppressed whether this combination was given before, at time of, or after infection. This is the first time in 30 years a new drug which is non-toxic and specific for a parasite enzyme pathway has been identified against leishmaniasis. Its prophylactic effect and ease of delivery suggest that DFMO should be tested in combination with other known, active compounds eg. Pentostam, and with new experimental ones, eg. allopurinol riboside.

FOREWORD

Citations of commercial organizations and trade names in this Report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

In conducting the research described in this report, the investigators adhered to the "Guide for Care and Use of Laboratory Animals", prepared by the Committees on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

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I. OBJECTIVE

To test the activity of experimental compounds against Leishmania braziliensis and L. mexicana subspecies in BALB/c mice as a secondary screening program to identify new agents against leishmaniasis in the Americas. These subspecies produce mucocutaneous and cutaneous disease in this animal model, respectively. Drug efficacy will be assessed by scoring lesions and examining the viscera for parasites. To test the activity of experimental drugs against L. donovani in BALB/c mice. Visceral leishmaniasis is a severe systemic disease and efficacy will be assessed by examining the liver and spleen for parasites.

II. BACKGROUND

Human leishmaniases are severely debilitating and affect about 100 million people. Their public health importance was recognized when the WHO Special Program included them among its 6 major diseases, and when the NIH-NIAID initiated its Collaborative Program for Training in Tropical Disease. Cutaneous disease in the Americas has always been a problem among U.S. Army personnel (1-2). In light of recent events in Central and South America, and the continued interest of the United States in the security of civilians and military in this hemisphere, cutaneous and mucocutaneous leishmaniasis, for which only microbistatic therapy is available, may become critical considerations in economic development and peace-keeping. Evidence also strongly suggests endemic areas of leishmaniasis to occur in southwestern U.S.A. (3-4), and the number of imported civilian cases continues to increase (5-7, personal observations).

Current therapy still involves the use of antimonials, arsenicals, and other toxic heavy metal drugs. Although these are generally effective against visceral leishmaniasis, they vary in efficacy against American cutaneous and mucocutaneous leishmaniasis (8-12). Rational approaches to chemotherapy are being developed (13-17). One of these uses liposomes to deliver high concentrations of drug into parasite-containing phagocytic vacuoles (17). Compounds under investigation also reflect increased awareness of differences between host and parasite in energy metabolism, membrane structure and function, subcellular location of critical enzymes, and metabolic pathways.

Although the 5-nitroimidazole, metronidazole, is not effective against kinetoplastids (18-20), 2-nitroimidazoles (Radanil) and 5-nitrofurans (Lampit) are (21,22). The 8-aminoquinoline lepidines, especially WR 6026, can be 700 times as effective as standard antimonials against experimental infections of L. donovani (23). These compounds probably function in disrupting the respiratory chain or pyrimidine biosynthesis (22).

A new compound, alpha D,L-difluromethylornithine (DFMO, RMI 71,782) alone and in combination with Bleomycin is a highly specific inhibitor of polyamine biosynthesis against bloodstream and central nervous system infections of African trypanosomes in mice (15,24,25). Here we report its remarkable efficacy against L. donovani infections in BALB/c mice.

Hemoflagellates synthesize large amounts of plasma membrane ergosterol in serum-free media (26). Ketoconazole and miconazole actively disrupt ergosterol synthesis in a variety of bacteria, fungi, and yeasts both in vivo and in vitro (27). An acid hydrolysate of ketoconazole also killed L. tropica major amastigotes and promastigotes in vitro (28). Recent data show three lipid targets for ketoconazole and miconazole (27). It is proposed that the shift from unsaturated, long-chain to saturated, short-chain fatty acids, and the general displacement of membrane lipids may be responsible for the in vitro effects of these imidazoles against leishmania (27) and Trypanosoma cruzi (29). Therefore, it was suggested that drugs which selectively change hemoflagellate lipid organization are reasonable targets for chemotherapy and should be tested in vivo. Here, we report the inefficacy of ketoconazole and its acid hydrolysate against L. donovani infections in BALB/c mice.

III. RESULTS

A. Aminoquinolines

During the second year of this contract, six aminoquinolines and 2 imidazoles have been tested for WRAIR against three subspecies of L. braziliensis, two L. mexicana, and L. donovani (Table 1). Their activity L. donovani and L. mexicana infections in BALB/c mice is summarized in Tables 2 through 6, and their mode of action in Fig. 1.

Six of these experimental compounds were as active as Pentostam in suppressing liver burdens (Table 7). The Pentostam Index (PI) for each compound was >1.00, based upon an Effective Dose (ED) 90 of 58 mkd x 5 for L. donovani and an ED 75 of >400 mkd x 15 for L. m. mexicana, respectively. Although liver parasite burdens were suppressed by the WRAIR aminoquinolines, spleen cultures were always positive. If the LD 50, PI and TI for these drugs are compared with Pentostam, it is clear that none is competitive (Table 7). The maximum tolerated dose for Pentostam is >600 mkd, whereas that for the WRAIR aminoquinolines is 10 to 125 mkd. The TI for Pentostam is 60 to 100x greater than any of these. Therefore, secondary screening in BALB/c mice indicates that none of the WRAIR drugs tested shows promise for further development.

B. Imidazoles

Although ketoconazole and its acid hydrolysate were reported effective against T. cruzi in vitro and in vivo (3,4), and

against amastigotes and promastigotes of Leishmania species in vitro (4), our tests in BALB/c mice against L. donovani are consistently negative (Table 8). These results agree with those of Chaia and H. Van den Bossch (pers. communication), and suggest that ergosterol synthesis is not a primary target for antileishmanial drugs. Based upon these preliminary data, we have not requested either imidazole for further testing.

C. Combination Chemotherapy

During the second year of this contract, several types of combination chemotherapy against cutaneous, mucocutaneous, and visceral infections in BALB/c mice were also tested. These included systemic and topical application of Pentostam against L. braziliensis infections, Pentostam and BCG against L. mexicana amazonensis infections, and DFMO in combination with Bleomycin against L. donovani infections.

1. Topical Pentostam

In the first set of experiments to test the effect of topical creams against two mucocutaneous species (Tables 9, 10), mice were infected as per our standard protocol with either L. b. panamensis or L. b. guyanensis. Pentostam at its ED 75 (400 Sb^{V} mkd x 10 or saline were given daily subcutaneously in 0.1 ml one month after lesion (50 mm diameter) development. Pentostam or placebo creams were applied daily in 0.1 ml directly to and evenly spread upon the lesion. All regimes using systemic Pentostam suppressed lesion development 77 to 87%. Neither Pentostam nor placebo cream alone were suppressive (Table 9, 10), Topical application of Pentostam to lesions did not improve systemic therapy. Differences in efficacy of treatment against ulcerated and non-ulcerated lesions were evaluated using the one-way analysis of variance at 90, 95, and 99% confidence levels. No significant differences could be detected.

2. BCG and Pentostam

BCG and Pentostam combinations were tested in Salvador, Bahia, Brazil. For this reason, BALB/c mice were infected intradermally into the right hind footpad instead of the naired tail base, with 0.1 ml infective promastigotes as per our standard protocol. Pentostam was administered SC daily starting either three days before (△), at time of (▲), or 3 weeks after infection (Figs. 2, 3). BCG was given one day prior to drug treatment whether mice were just infected (□——) or had three-week developed lesions (■– – –). Suppression was measured by comparing the difference in footpad swelling between those mice infected with leishmania and those injected with saline. Differences were measured every 3 to 4 days throughout the treatment period. At necropsy, liver and spleen, draining lymph nodes, and lesions were removed for histopathology.

Well-developed (10 mm diameter) lesions (■– – –) were

more sensitive to BCG/Pentostam than were developing ones (□—○).

Male mice were more sensitive to infection with L. m. amazonensis and to treatment with BCG/Pentostam than were females (Figs. 2, 3). Four weeks after treatment, lesions were still regressing. Male mice remained negative longer, but all mice eventually relapsed and lesions reappeared (Fig. 2).

These results suggest that BCG in combination with Pentostam may enhance the immune response and promote faster resolution of lesions during treatment. However, the final outcome of infection is not altered and no cures were obtained.

3. Polyamine Inhibitors

Several investigators have shown the synergistic effect of the polyamine inhibitor DFMO and the antineoplastic glycopeptide Bleomycin against bloodstream and central nervous system infections of Trypanosoma species in mice (15,24,25).

In a series of pilot experiments, our data show that liver burdens of mice infected with L. donovani and treated with 1% DFMO in the drinking water before or at time of infection in combination with Bleomycin on the day of infection, were suppressed 91% and 87%, respectively (Tables 11, 12). Neither compound alone was suppressive (Table 11). The treatment did not cure mice, as measured by liver impressions and cultures (Tables 11, 12), but the treatment was competitive with Pentostam. Liver burdens were suppressed 46% even when treatment was begun 3 days after infection (Table 12). This may be biologically significant, although the variation was considerable.

It is worth noting that this is the first time in more than 30 years that any treatment has been equal to that of the antimonials. That DFMO is also parasite specific, non-toxic, and easy to deliver suggests that combination chemotherapy using this drug should be further explored.

IV. DISCUSSION and CONCLUSIONS

A. Aminoquinolines

From 1980 - 1982, five 8-aminoquinolines, two imidazoles, and the antimalarial primaquine phosphate were tested for efficacy as antileishmanial agents. All have low Therapeutic and Pentostam Indices (Table 7), and three of the aminoquinolines caused mild to moderately toxic symptoms including i)bleeding at injection site, (ii tachynea, iii) internal hemolysis, and iv) hyperactivity. These are known side effects of aminoquinolines (30).

The rational for testing aminoquinolines is that those groups with substitutions at the 3 and 5 methyl groups (lepidines) are especially active against leishmania in vivo (23) and

in vitro (13). Although their mode of action is not yet understood, it is suggested that they interfer with mitochondrial respiratory ubiquinones (Figure 1, 24). The 8-aminoquinoline Moxipraquine (31) was suppressive against *T. cruzi*, *L. mexicana* and *L. braziliensis* as long as treatment was applied, but cures were never achieved (31). This compound reached clinical trials before it was discovered to be teratogenic in rats and rabbits. At that time its development as an alternative to antimonials was discontinued (31). To date then, none of the aminoquinolines tested, have Therapeutic Indices competitive with pentavalent antimonials (31, Table 7).

B. Imidazoles

Ketoconazole and its acid hydrolysate were also inactive against visceral leishmaniasis in BALB/c mice (Table 8). As mentioned before, hemoflagellates synthesize large amounts of plasma membrane ergosterol in serum-free medium. Ketoconazole and miconazole actively disrupt ergosterol synthesis in a variety of bacteria, fungi, and yeasts both *in vitro* and *in vivo* (14). An acid hydrolysate of ketoconazole killed *L. tropica* amastigotes and promastigotes *in vivo* (15). Recently, ketoconazole was shown to inhibit sterol biosynthesis *in vitro*, both in the presence and absence of serum, at the level of demethylation (Berman, J.D., G.G. Holz, Jr., and D.H. Beach, Leishmania sterol biosynthesis is inhibited by ketoconazole Abstract 16, 36th Ann. Meeting Soc. Protozool., Pace Univ., New York City, 20-24 June, 1983). However, the intracellular uptake and inhibition of amastigotes by this drug was not tested. Under natural conditions within a host, cholesterol is probably preferentially used by these protozoa. Our negative data *in vivo* for this drug would tend to support this hypothesis, and indicate that ergosterol is not a promising target for antileishmanial therapy.

More recently, oral ketoconazole has been used at high doses once or twice daily for 3 months to treat human cutaneous and mucocutaneous leishmaniasis (33,34). Cures were claimed, although the follow-up was only for several months. Some patients experienced dizziness and somnolence. Neither the Therapeutic Index nor the treatment regime using ketoconazole is competitive with Pentostam, and the manufacturers have decided it is not a promising alternative to antimonial therapy (H. Van den Bossche, pers. commun.).

C. Combination Chemotherapy

Combination chemotherapy has been used successfully against blood and tissue sporozoa, eg. Plasmodium and Toxoplasma species (22). Until recently, however, it was rarely applied successfully to infections caused by trypanosomatids (15,32). By combining leads in the development of rational targets for chemotherapy

with known active compounds against trypanosomiasis"), several new avenues of treating leishmaniasis have become apparent.

1. Topical Pentostam

The rationale for topical treatment of cutaneous or mucocutaneous leishmaniasis with Pentostam ointment was to test whether the same drug dose alone delivered directly to the target tissue could cause lesion resolution and cure infections. Topical application would avoid systemic toxicity, since the amount finally reaching subepidermal capillaries would be sufficiently diluted after diffusion, binding, or forming depots within the stratum corneum (sc), epidermis, dermis, and skin glands (35). It also might effectively lower the drug concentration necessary to cure, since percutaneous absorption and delivery to the macrophage should be more efficient. Vehicles with affinity for the sc should enhance drug delivery to the hydrated subepidermal capillaries from which macrophages migrate after sandflies probe them for a blood meal.

In this study, Pentostam was applied once daily as a water-in-oil cream (37.5% Sb^V: 20 g aquaphore) 5 days per week for 3 weeks as per our standard protocol. The cream was gritty, and somewhat sticky when spread over the lesion; the placebo cream spread easily and appeared to be more readily absorbed. Enhancing penetrants eg. DMSO were not used. Water-in-oil creams usually easily permeate the sc, delivering compound to the epidermis. Pentostam is soluble in water and should become highly concentrated within the sc as the water evaporates. The hydrated epidermis could then keep Pentostam in solution for delivery to the target tissue and its macrophages.

$^{125}Sb^V$ sodium stibogluconate is readily taken up *in vitro* by amastigotes (36). However, it is not known whether pentavalent antimony is metabolized to its active trivalent form by the skin. Nothing is known about Pentostam metabolism in the skin, and very little about its conversion from Sb^V to Sb^{III} *in vivo*. Steroid hormones are catabolized by skin slices *in vitro* both to active and inactive metabolites (35). Therefore, it is possible that Pentostam is catabolized by the skin, but through some route which renders most of the drug harmless. If reduction of Sb^V requires reduction by liver enzymes, then topical application of Pentostam would be futile. If, however, macrophages reduce the drug, then activity should be detected. Our data indicate that Pentostam cream alone is unable to suppress lesions of *L. braziliensis* subspecies *in vivo*. When systemic Pentostam is given alone or in combination with Pentostam cream, suppression occurs (Table 9, 10). These data indicate that reduction of Sb^V to its active metabolites occurs systemically.

Drug delivery and regimes may have contributed to inefficacy. In two previous studies, a kerolytic base and penetrant were used to deliver chlorpromazine or imidazoles to cutaneous lesions caused by *L. tropica* or *L. m. amazonensis* (37, 38). These creams were applied either 3x daily for one month or 2x

daily for 20 days, whereas we applied the creams once daily for 15 days. However, the final outcome of each of these studies does not differ significantly from ours. The three patients treated topically with chlorpromazine showed improvement (37), whereas BALB/c mice and the 8 patients treated with imidazoles did not (38).

Together these data indicate that much more needs to be known about percutaneous absorption and drug metabolism of Pentostam by the skin if topical chemotherapy is to succeed.

2. BCG and Pentostam

The rationale for using BCG with Pentostam against leishmaniasis is to enhance the host immune response concurrent with treatment. BCG is used as an adjuvant to immunize against bacterial infections, by nonspecifically enhancing cell-mediated immunity through immunostimulation of macrophages and T-lymphocyte subsets (39). Since mycobacteria cell walls cross-react with L. donovani (40), it was thought that immunostimulation of a host with BCG prior to infection with L. mexicana amazonensis, might increase the efficacy of Pentostam. Subspecies of L. mexicana in the New World are associated with diffuse cutaneous leishmaniasis (DCL), especially in L. m. amazonensis infections. In Para State, Brazil, 41% of patients with L. m. amazonensis progress to DCL (41).

Previous studies using BCG against cutaneous leishmaniasis are inconclusive. Mice pretreated with BCG were better able to control L. tropica infections, as measured by reduction of lesion size and metastasis to viscera (42), but BCG was unable to alter either the course of infection or the immunological response of C3H mice to infection with L. mexicana (43).

Unlike the latter authors, we observed a marked decrease in lesion size when BCG was combined with Pentostam (Tables 3 & 4). Pentostam is known to accumulate both in vitro within phagolysosomes and leishmania (36), and is thought to inhibit phosphofructokinase and pyruvate kinase, two enzymes important for glycolysis (22). In kinetoplastids, these enzymes are compartmentalized into the glycosome (44, 45). Ultrastructural evidence indicates that pentostam treatment of L. mexicana infected hamsters causes these organelles to disappear (19). Therefore, the known specificity of Pentostam for an essential pathway localized in an organelle in leishmania, may account for its efficacy in reducing lesions when combined with BCG. Neither BCG nor levamisole alone altered L. mexicana infections (43). Perhaps combining either of these immunostimulators with specific antileishmanial drugs should have been explored by the authors.

The ultimate failure to cure L. mexicana amazonensis infections in our system may have been due to the fact that leishmania can obtain sufficient energy from the B-oxidation of fatty acids

and the hexose monophosphate shunt (45), and to replace glycolysis. If BCG, Pentostam, and DFMO were used (C.3.), complete cures might have been obtained because: i) BCG enhances immunity, ii) DFMO prevents an essential biosynthetic pathway for growth, and iii) Pentostam removes a major source of energy.

As more is learned about the cross-reaction of BCG with leishmania, essential leishmania metabolic pathways and mode of action of known antileishmanial agents, a better understanding of how to use BCG alone and in combination with other drugs should be possible. We recommend the potential of this immunostimulator in combination with other drugs be explored.

3. Polyamine Inhibitors

The rationale for combination chemotherapy using the polyamine inhibitor DFMO and the antineoplastic glycopeptide Bleomycin against leishmania is based upon its known mode of action against bloodstream and central nervous system infections of trypanosomes (15, 23, 24).

Trypanosomes require ornithine for biosynthesis of the polyamines spermidine and spermine, which are necessary for cell division, differentiation, and protein synthesis. Unlike their host, trypanosomes must sequentially use the pathway for polyamine biosynthesis (Fig. 4). The rate limiting step in this sequence is controlled by the enzyme ornithine decarboxylase (ODC). DFMO structurally resembles ornithine. Therefore, when DFMO enters the system, ornithine decarboxylase gets sidetracked into decarboxylating a useless compound. No polyamines are formed, and cell division is stopped in G_1 (14).

Bleomycin (Fig. 4) contains a variety of polyamine side chains. Trypanosomes starved for polyamines by DFMO will preferentially take up Bleomycin. Bleomycin then binds to the PO_4 of nuclear DNA, causing its strands to break. The combination of DFMO and Bleomycin then, rapidly and specifically kills hemoflagellates (Tables 13, 14).

Our data (Tables 11 & 12) suggest that combinations of DFMO with known (Fig. 5) and promising antileishmanial compounds should be tested. If the best 8-aminoquinolines (WR 6026), 5-nitrofurans (Lampit), 2-nitroimidazoles (Radanil), and polyene antibiotics (amphotericin B) also act synergistically with DFMO, then less toxic doses might be effective enough to provide alternate therapy against unusual or resistant New and Old World leishmaniases. In combination with this drug, the efficacy of allopurinol riboside, pentamidine and pentostam might also be improved. Therefore, we recommend that combinations of DFMO with a selection of known and promising compounds be tested in each of our BALB/c models of leishmaniasis.

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Table 1. Summary of Compounds Tested Against Leishmania 1981-82.

Compounds	Species					
	L. braziliensis			L. mexicana		L. donovani
Aminoquinolines	LTB-05	CUMC 1	WR-120	WR-303	WR-183	WR-130
WR-211-666			±	±	±	+
227-495			±	±	±	+
241-317			±	±	±	+
242-511	(+)	(+)	+		(+)	+
219-423	(+)	+	+		(+)	+
2975	(+)	+	+		(+)	+
Imidazoles						
WR-248-310						+
249-27						+

L. braziliensis complex	L. mexicana complex
L. b. braziliensis (LTB-05)	L. m. amazonensis (WR-303)
L. b. guyanensis (MHOM/SR/80/CUMC 1	L. m. mexicana (WR-183)
L. b. panamensis (WR-120)	L. donovani Khartoum (WR-130)

(+) being tested + = tested ± = needs repeating; toxicity.

Table 2

COMPARATIVE ACTIVITY OF PENTOSTAM (BJ 58563) AND 6 WR-COMPOUNDS ON *Leishmania donovani* Khartoum IN BALB/C MICE

Compound	Type Test		mkd x 5[1]	Animals[2]	Time Treated Weeks	LDUs[3] Exptl.	LDUs[3] Pentostam	LDUs[3] Control	P Index*
WR 219423	Toxicity		1	0/3	1				
			4	0/3					
			8	0/3					
			12	0/3					
			16	2/3					
	Exptl.	A	9	0/5	1	0.00	3.00 ± 7	1095 ± 330	7.8
			12	0/5		0.00			
		P		0/5		5.00 ±6	6.00 ± 10	200 ± 158	
			(x 10)	0/5	2	4.00 ±6	7.00 ± 8	1001 ± 206	
WR-2975	Toxicity		50	0/2	1				
			100	0/6	1,2				
			150	2/3	1				
			200	3/3					
	Exptl.	A	75	0/4	1	1.00 ±2			1.4
			100	0/3		0.00			
		P		0/3		2.00 ±4			
			(x 10)	0/5	2	0.50 ±1			
WR-211666	Exptl.	A	24	0/4	1	1.00 ±2	1.20 ± 2	184 ± 110	3.1
WR-227495			12	0/5		0.40 ±1			5.8
WR-241317			6	0/5		0.00			11.8
WR-242511		P	10	0/5		2.70 ± 2	0.30 ± 1	64 ± 77	7.2
			15	0/5		5.30 ± 7			

A = amastigotes, P = fully-infective promastigotes grown in Schneider's drosophila medium + 15% HIFCS (Sterile Systems, Inc., Logan, Utah)

[1] milligrams/kg/day for 5 days; (x 10) = treated two weeks, 5 days each.

[2] number of deaths/total number of animals

[3] ratio (amastigotes in liver/cell nuclei) x mg liver weight

*Pentostam Index >1 = significantly greater suppression

Table 3

LEISHMANIASIS

Comparison of the suppressive effect of Pentostam and various compounds on Leishmania m. mexicana 183 in BALB/c ByJ mice

Exp. No. 3 (1-3) Date 4/22/82 (4-8) Route 1 (9) Regimen 2 (10) Type Test Exptl. (11) Animal 3 (12) *Strain 3a (13)

7/02/82 Amastigotes

1. SC 4. IM 2. IP 7. GAVAGE

1. 2X4D 2. 1 x 5D 3. 1 x 10D 4. 1 x 15D

1. HAMSTER 2. DOG 3. Mouse

1. KHARTOUM 2. brasiliensis 3. mexicana

COMPOUND NO.	MG/KG/DA	ANIMALS TOT	ANIMALS EXP	ANIMALS TOX	Mean Lesion Necropsy Wt Culture		Mean Lesion Size (mm^2) Weeks 2	4	8	% SUPPRESSION Weeks 2	4	8	SIG. 1. YES 2. NO	† Pentostam INDEX PI	TI
14 - 21	22 - 27	28 - 30			mg 31 - 35	+/-	Total 36 - 44			45 - 49			50	PI 51 - 57	TI
WR 211-666	15	6	6	0	534	2/2	171	239	374	31	26	25	2	1.06	1.4
	25	6	0	6	—		—			—					
	30	3	0	3	—		—			—					
WR 227-495	10	6	6	0	348	2/2	141	180	312	43	44	38	2	3.80	1.8
	15	6	0	6	—										
	25	3	0	3	—										
WR 242-511	15	6	1	5	578	1/2	150	200	275	39	38	45	2	0.00	<1.00
	20	6	0	6	—										
WR 219-423	10	6	1	5	411	1/2	71	157	300	71	51	40	2	0.00	<1.00
	15	6	0	6	—										
Pentostam	400	6	5	1	202	2/2	82	116	222	67	64	56	—	-	16.00
	800	6	4	2	79	2/2	60	81	169	76	75	66	—	-	16.00
Saline	0.1 cc	6	6	0	341	2/2	247	323	502	0	0	0	2		

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* 2. a. L.b.panamensis (WR 120) b. L.b.guyanensis (CUMC 1) c. L.b.brasiliensis (LTB 05) d. L.b.b. (type 79-80) M 1287

3. a. L.m.mexicana (WR 183) b. L.m.amazonensis (WR 303) c. L.m.mexicana (Type L-11)

Table 4

LEISHMANIASIS

Comparison of the suppressive effect of Pentostam and various compounds on Leishmania mexicana in the BALB/cBYJ mouse

Exp. No. 2 (1-3) Date 12/22/81 (4-8) 4/07/82 Route 1 (9) 1. SC 4. IM 2. IP 7. GAVAGE Regimen 2 (10) 1. 2X4D 2. 1x 10D Type Test Expt'l (11) Animal 3 (12) 1. HAMSTER 2. DOG 3. Mouse Strain *3 a (13) 1. KHARTOUM 2. brasiliensis 3. mexicana

COMPOUND NO.	MG/KG/DA	ANIMALS TCT	ANIMALS EXP	ANIMALS TOX	Necropsy Lesion Wt. / Culture		Necropsy MEAN Lesion Size (Weeks after treat.)			% SUPPRESSION Weeks			SIG. 1. YES 2. NO	PENTOSTAM INDEX
14-21	22-27	28-30			mg.	+/-	1	3	7	1	3	7	50	51-57
Saline	0.1 cc	5	5	0	536	2/2	115	147	213	0	0	0		
BJ 58563	100 x 10D	5	5	0	350	2/2	105	132	174	9	10	18		
	200 x 10D	4	4	0	642	2/2	96	126	177	17	14	17		
	400 x 10D	5	5	0	314	1/2	91	101	127	21	31	40		
WR 242-511	3.0 x 10D	5	5	0	515	1/1	117	147	192	0	0	10	2	
	6.1 x 10D	5	5	0	408	1/2	108	145	178	6	1	16	2	
	12.2 x 10D	4	4	0	541	2/2	101	117	168	12	20	21	2	
WR 219-423	2.5 x 10D	5	5	0	632	0/2	100	143	188	13	03	12	2	
	5.0 x 10D	5	5	0	521	1/2	110	136	168	04	06	27	2	
	9.9 x 10D	5	5	0	462	0/2	99	123	162	14	16	24	2	

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2. a. L. b. panamensis (WR 120)
b. L. b. guyanensis (CUMC 1)
c. L. b. brasiliensis (LIB 05)
d. L. b. brasiliensis (Type specimen, M1287)

*3.a. L. m. mexicana (WR 183)
b. L. m. amazonensis
c. L. m. mexicana (Type specimen, L_{11})

Table 5

LEISHMANIASIS

Comparison of the suppressive effect of Pentostam and various compounds on Leishmania m. mexicana in BALB/c ByJ mice

Exp. No. 2 (1-3) Date 3/02/82 (4-8) Route 1 (9) Regimen 4 (10) Type Test Exptl (11) Animal 3 (12) *Strain 3a (13)

6/11/82 Promastigotes

1. SC 4. IM 2. IP 7. GAVAGE

1. 2X4D 2. 1 x 5D 3. 1 x 10D 4. 1 x 15D

1. HAMSTER 2. DOG 3. Mouse

1. KHARTOUM 2. brasiliensis 3. mexicana

COMPOUND NO.	MG/KG/DA	ANIMALS TOT	EXP	TOX	Mean Lesion Necropsy Wt Culture		Mean Lesion Size (mm^2) Weeks 2	4	8	% SUPPRESSION Weeks 2	4	8	SIG. 1. YES 2. NO	! Pentostam INDEX Spleen Culture
14-21	22-27	28-30			mg 31-35	+/Total	36-44			45-49			50	51-57 +/Total
WR 211-666	15	6	6	0	387	2/2	128	203	258	45	44	39	2	1.06
WR 227-495	10	6	6	0	435	2/2	142	237	351	39	34	17	2	1.00 (0/1)
WR 242-511	15	3	3	0	578	1/2	448	655	1047	0	0	0	2	(1/1)
	10	3	3	0	639	2/2	149	197	258	36	45	39	2	2.5
WR 219-423	10	3	3	0	364	2/2	124	147	191	46	59	55	2	
	08	3	3	0	317	2/2	146	165	180	37	54	57	2	6.2
Pentostam	400	4	4	0	147	2/2	88	100	108	[illegible]	72	74	1	(1/1)
Saline	0.1 cc	4	4	0	658	2/2	231	360	422	0	0	0		

WRAMC FORM 1 JUN 78 1468

* 2. a. L.b.panamensis (WR 120) b. L.b.guyanensis (CUMC 1) c. L.b.brasiliensis (LTB 05) d. L.b.b. (type 3. a. L.m.mexicana (WR 183) b. L.m.amazonensis (WR 303) c. L.m.mexicana (Type L-11) 79-80 M 1287

Table 6

LEISHMANIASIS

Comparison of the suppressive effect of Pentostam and various compounds on Leishmania m. mexicana in BALB/c ByJ mice

Exp. No. 4 (1-3) Date 4/22/82 (4-8) Route 1 (9) Regimen 4 (10) Type Test Exptl. (11) Animal 3 (12) *Strain 3a (13)

7/23/82 Amastigotes

1. SC 4. IM 2. IP 7. GAVAGE

1. 2X4D 2. 1 X 5D 3. 1 x 10D 4. 1 x 15D

1. HAMSTER 2. DOG 3. Mouse

1. KHARTOUM 2. brasiliensis 3. mexicana

COMPOUND NO.	MG/KG/DA	ANIMALS TOT	ANIMALS EXP	ANIMALS TOX	Mean Lesion Necropsy Wt / Culture	Mean Lesion Size (mm^2) Weeks 5	Mean Lesion Size (mm^2) Weeks 7	% SUPPRESSION Weeks 5	% SUPPRESSION Weeks 7	SIG. 1. YES 2. NO	! Pentostam INDEX Spleen Culture
14-21	22-27	28-30			mg 31-35 +/	Total 36-44		45-49		50	51-57 +/Total
Saline	0.1 cc	3	3	0	544	150	177	0	0	2	-
BJ 58563	400	5	5	0	166 —	63	66	58	62	1	-
WR 2116-66	15	5	5	0	514 2/2	143	180	2	0	2	0.00 (2/2)
WR 227-495	10	5	5	0	653 2/2	125	158	17	11	2	0.50 (2/2)
WR 219-423	12	5	1	4	549 1/1	119	138	21	22	2	0.00 (1/1)

WRAMC FORM 1 JUN 78 1468

* 2. a. L.b.panamensis (WR 120) b. L.b.guyanensis (CUMC 1) c. L.b.brasiliensis (LTB 05) d. L.b.b. (type 79-80

3. a. L.m.mexicana (WR 183) b. L.m.amazonensis (WR 303) c. L.m.mexicana (Type L-11)

M 1287

Table 7. Comparison of Pentostam and Therapeutic Indices for all Drugs Tested 1981 - 1982.

Compound Tested	LD50 (mkd)	Pentostam Index		Therapeutic Index
		Visceral	Cutaneous/Mucocutaneous	
Pentostam Sb^V	600	-	-	160.00
WR- 2975	125	1.40	< 1.00	2.00
211-666	24	3.10	1.06	1.00
227-495	18	5.80	1.00	1.
219-423	14	7.80	6.20	1.55
242-511	12	7.20	2.50	1.20
241-317	10	11.80	< 1.00	1.66

TABLE 8

LEISHMANIASIS

Comparison of the suppressive effect of Pentostam & various compounds on Leishmania donovini in the mouse

Exp. No. 1 (1-3) Date 12/02/81 (4-8) Route 1 (9) Regimen 2 (10) Type Test Expt. (11) Animal 3 (12) Strain ? (13)

1. SC 4. IM
2. IP 7. GAVAGE

1. 2X4D 4. 1x10 D
2. 1x5 D 5. 1x14 D
3. 1x6 D

1. HAMSTER
2. DOG
3. MOUSE

1. KHARTOUM
2. SUDAN
3. CHAGASI

COMPOUND NO.	MG/KG/DA	ANIMALS TOT	ANIMALS EXP	ANIMALS TOX	% WT. CHANGE	MEAN # PARASITES/LIVER	% SUPPRESSION	SIG. 1. YES 2 NO	PENTOSTAM INDEX
14 - 21	22 - 27	28 - 30			31 - 35	36 - 44	45 - 49	50	51 - 57
Saline		6	6	0	None	1389 ± 373	0		
BJ 58410	58	2	2	0	"	1598 ± 15?	0	2	
	232	4	4	0	"	1225 ± 155	0	2	
BJ 84232	58	5	5	0	"	1617 ± 598	0	2	
	232	4	4	0	"	1454 ± 70	0	2	
BJ 58563	104	5	5	0	"	704 ± 444	49	1	
	208	5	5	0	"	320 ± 115	77	1	

Table 9

LEISHMANIASIS

Comparison of the suppressive effect of Pentostm and various compounds on Leishmania brasiliensis guyanensis in BALB/cBYJ Mice

Exp. No. 1 (1-3) Date 2/11/82 (4-8) 4/01/82 Route 1 (9) 1. SC 4. IM 2. IP 7. GAVAGE Regimen 4 (10) 1. 2X4D 4. 2 x 15D Type Test Expt'l (11) Animal 3 (12) 1. HAMSTER 2. DOG 3. Mouse Strain 2b (13) 1. KHARTOUM *2. brasiliensis 3. mexicana

COMPOUND NO.	MG/KG/DA 400 Sb[V]	ANIMALS TOT	ANIMALS EXP	ANIMALS TOX	Mean Lesion Wts. mg. at Necropsy	MEAN Lesion Size (mm^2) Weeks 2	4	8	% SUPPRESSION Weeks 2	4	8	SIG. 1. YES 2. NO	PENTOSTAM INDEX
14 - 21	22 - 27	28 - 30			31 - 35	36 - 44			45 - 49			50	51 - 57
Saline	0.1 cc	7	7	0	155	153	163	169	0	0	0		
BJ 58563	Cream alone	8	8	0	310	155	169	175	0	0	0	2	
Placebo	Cream alone	8	8	0	191	122	108	122	0	0	0	2	
BJ 58563	Solution only	8	8	0	172	73	39	23	27	61	77		
BJ 58563	Cream + Soln.	8	8	0	100	74	29	19	26	71	81	2	
BJ 58563	PlaCrm + Soln	8	8	0	78	54	41	14	46	59	86	1 (originally)	

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*2.a. L. b. panamensis (WR 120)
b. L. b. guyanensis (CUMC 1)
c. L. b. brasiliensis (type 3)28/

Table 10

LEISHMANIASIS

Comparison of the suppressive affect of Pentostm and various compounds on Leishmania brasiliensis panamensis in BALB/cBYJ mice

Exp. No. 1 (1-3) Date 2/11/82 (4-8) 4/01/82 Route 1 (9) 1. SC 4. IM 2. IP 7. GAVAGE Regimen 4 (10) 1. 2X4D 2. 1 x 10D 3. 1 x 5D 4. 1 x 15D Type Test Expt'l (11) Animal 3 (12) 1. HAMSTER 2. DOG 3. Mouse Strain 2a (13) 1. KHARTOUM * 2. brasiliensis 3. mexicana

COMPOUND NO.	MG/KG/DA 40CSb[V]	ANIMALS T O T	E X P	T O X	Mean Lesion wt. mg. at Necropsy	MEAN Lesion Size (mm^2) Weeks 2	4	8	% SUPPRESSION Weeks 2	4	8	NO. 1. YES 2. NO	PENTOSTAM INDEX
14 - 21	22 - 27	28 - 30			31 - 35	36-44			45-49			50	51 - 57
Saline	0.1 cc	7	7	0	179	133	138	177	0	0	0		
BJ 58563	Cream only	8	8	0	304	153	159	239	0	0	0	2	
Placebo	Cream only	7	7	0	241	145	179	258	0	0	0	2	
BJ 58563	Solution only	8	8	0	30	76	45	09	24	55	91		
BJ 58563	Cream + Soln	8	8	0	45	73	39	14	27	61	86	2	
BJ 58563	PlaCrm + Soln	7	7	0	32	42	33	10	51	67	90	2	

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* 2.a. L. b. panamensis (WR 120)
b. L. b. guyanensis (CUMC 1)
c. L. b. braziliensis (LTB 05)
d. L. b. brasiliensis (Type specimen, M 1287)

Table 11. Synergistic Effect of DFMO and Bleomycin on Leishmania donovani Infections in BALB/c Mice.

Treatment*	Dose (mkd)	Liver Burdens Mean ± SD	Percent Suppression	Cultures +/Total
Control		308 ± 48	0	3/3
Pentostam	140	0	100	2/2
DFMO + Bleomycin	3	41 ± 94	87	2/3
DFMO Alone		260 ± 238	16	2/2
Bleomycin Alone	3	331 ± 148	0	2/2

*DFMO = 1% drinking water 24 hours before infection; Bleomycin given SC at time of infection. Both continued for 5 days as per standard protocol.

Table 12. Prophylactic Effect of DFMO and Bleomycin on Leishmania donovani Infections in BALB/c mice.

Treatment*	Dose (mkd)	Liver Burdens Mean ± SD	Percent Suppression	Cultures +/Total
Before				
Control	-	304 ± 174	0	3/3
Pentostam	140	0	100	2/3
DFMO + Bleo	3	14 ± 22	91	2/3
After				
Control		820 ± 556	0	3/3
Pentostam	140	0	100	3/3
DFMO + Bleo	3	374 ± 399	46	3/3

Mice = 5/group *DFMO = 1% in drinking water; Bleomycin = subcutaneously. Both given 3 days prior to or after infection, continued for 7 days.

TABLE 13. SUMMARY: DFMO/BLEOMYCIN SYNERGY

DFMO

1. UPTAKE OF ORNITHINE ANALOGUE
2. DEPLETION OF ORNITHINE DECARBOXYLASE
3. NO POLYAMINES SYNTHESIZED
4. NO CELL DIVISION. STOPPED IN G_1

BLEOMYCIN

1. FACILITATED UPTAKE DUE TO POLYAMINE SIDECHAINS
2. DNA VULNERABLE TO STRAND BREAKAGE
3. DEATH OF TRYPANOSOMES AND LEISHMANIA

TABLE 14. SUMMARY: SELECTIVITY, ADVANTAGES, AND USES OF DFMO

SELECTIVE ACTION OF DFMO

UPTAKE BY RAPIDLY DIVIDING CELLS
PATHWAY ESSENTIAL FOR PARASITE

ADVANTAGES

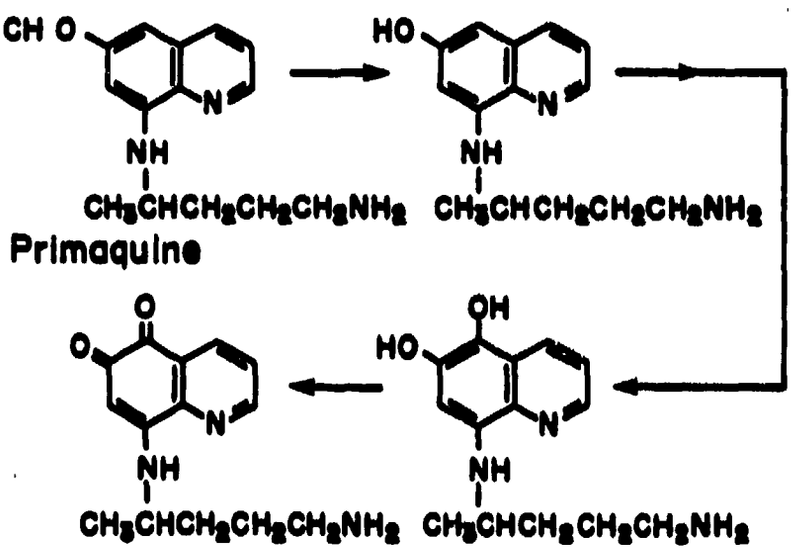
NON-TOXIC
EASE OF DELIVERY - DRINKING H_2O

USES

PROPHYLAXIS
TREATMENT

FIGURE 1. BIOCHEMICAL MECHANISMS OF DRUG ACTION: I ENERGY METABOLISM

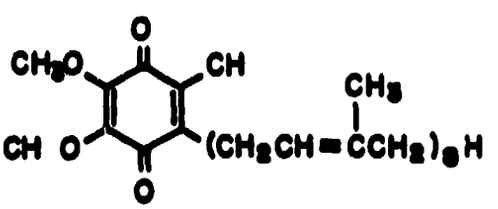
A. Metabolism of 8-Aminoquinolines (Primaquine, WR6026)



Primaquine

5,6-Quinoline diquinone

B. Mode of Action: Respiratory Chain Disruption



Ubiquinone 8

Figure 2.

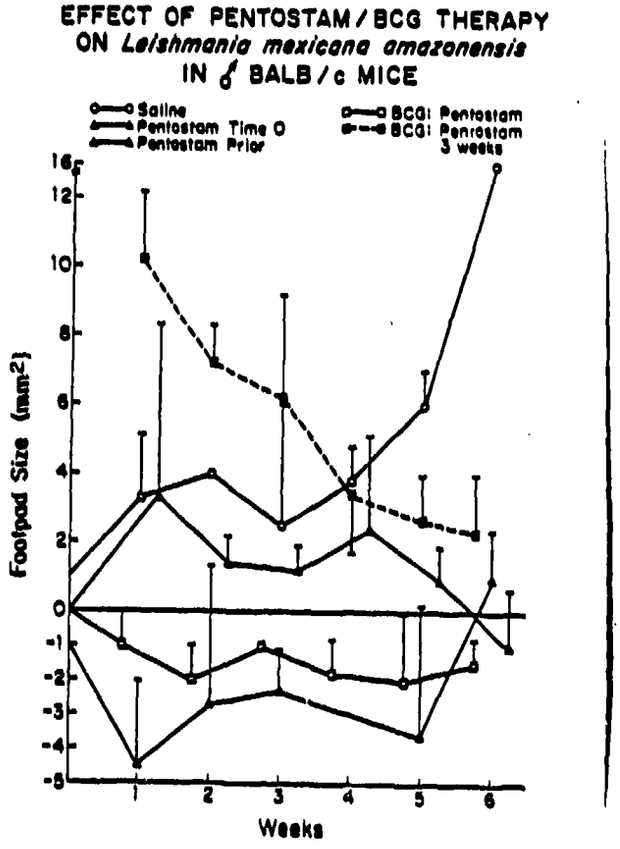


Figure 3.

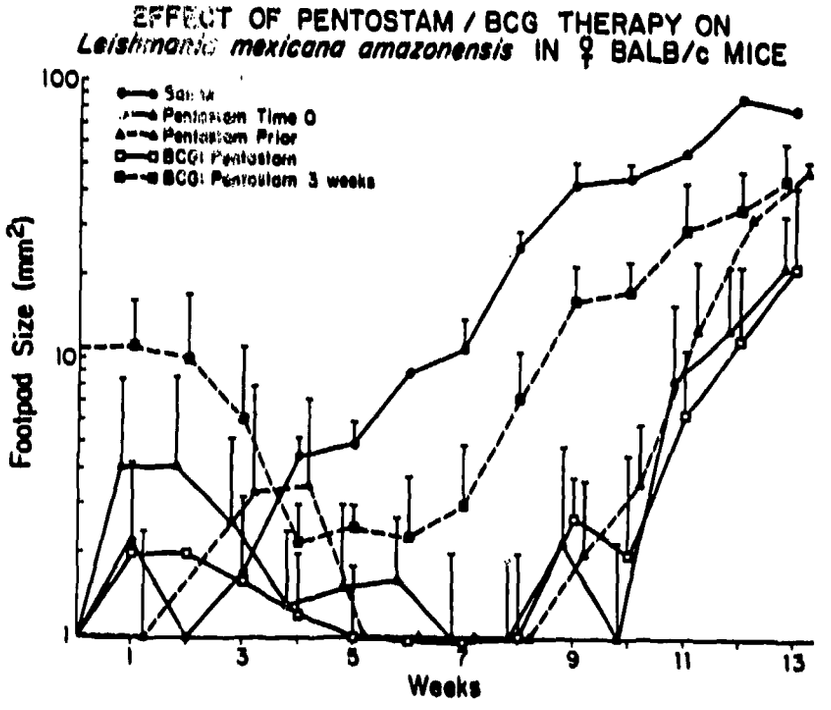
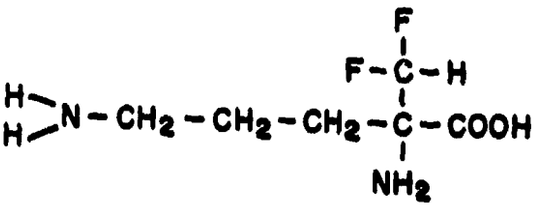


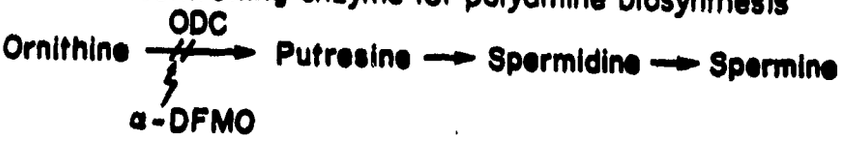
FIGURE 4. BIOCHEMICAL MECHANISMS OF DRUG ACTION:

Inhibitors of Polyamine Biosynthesis

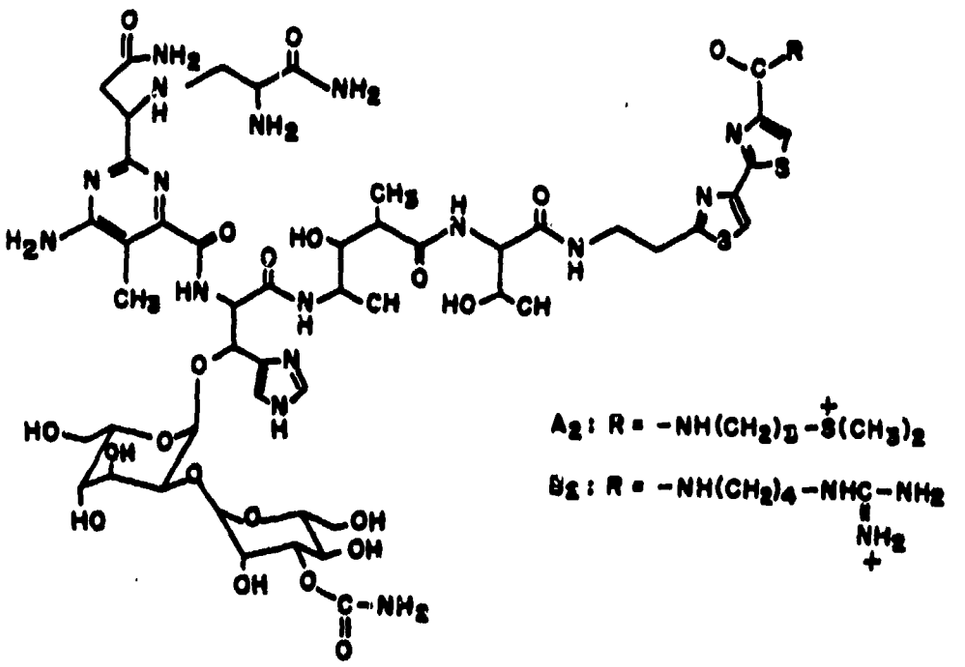
1. Alpha-difluoromethylornithine (α-DFMO)



Mode of action: Inhibits ornithine decarboxylase (ODC), the rate-controlling enzyme for polyamine biosynthesis



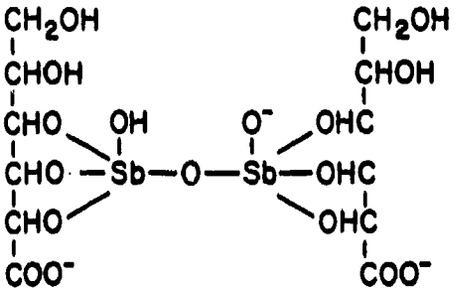
2. Bleomycin



Mode of action: Binds to DNA

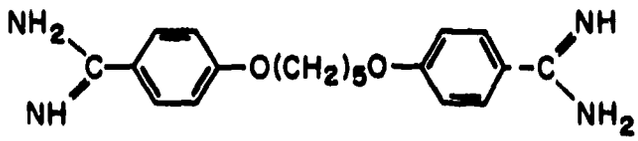
FIGURE 5. KNOWN DRUGS AGAINST HEMOFLAGELLATES

Pentavalent Antimonials



Sodium Stibogluconate
(Pentostam)

Aromatic Diamidines



Pentamidine

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